

*REMARKS/ARGUMENTS**The Present Invention and the Pending Claims*

The present invention provides a method for selectively increasing glutamate and/or aspartate release in a central nervous system (CNS) locus in a site-specific manner. Claims 1-6 currently are pending.

Summary of the Office Action

The Office Action rejects claims 1-6 under 35 U.S.C. § 103(a) as being obvious over Tice et al. (U.S. Patent 5,360,610) in view of Heya et al. (EP 0 256 726 B1). Reconsideration of the pending claims is respectfully requested.

Discussion of the Obviousness Rejection

Claim 1 allegedly is obvious over Tice et al. in view of Heya et al. Tice et al. allegedly teaches a method of implanting microspheres directly into the central nervous system. The microspheres disclosed by Tice et al. are composed of biodegradable polymers and bioactive agents, including neurotransmitters, neuropeptides, and neurotrophic factors. Heya et al. allegedly discloses microencapsulated TRH with some of the same polymers as disclosed in Tice et al. to construct their microencapsulated formulations.

The obviousness rejection is respectfully traversed because the cited references (alone or in combination) do not disclose or reasonably suggest the present invention as recited in the pending claims.

The Examiner relies on Heya et al. and the disclosure of the use of a microcapsule for delivery of TRH to the central nervous system. Although Heya et al. discloses administration of TRH to the CNS, it does not teach regulation of endogenous molecules through the use of TRH at all, let alone the increase of glutamate and/or aspartate by administering TRH. If delivery of the microcapsule/microstructure is not site-specific, as required by the pending claims, then it cannot be assumed that increasing glutamate and/or aspartate is inherent in the mode of administration disclosed by Heya et al.

Tice et al., on the other hand, discloses locally administering compounds to the CNS but for the purpose of stimulating nerve fiber growth. The administration of TRH in any form is *not* disclosed. Therefore, starting with the disclosure of Tice et al., as the Examiner has suggested, one of ordinary skill in the art would not necessarily be led to the teachings of Heya et al., since the two references are directed to completely different inventions. There simply is no nexus between the inventive concepts of Tice et al. and Heya et al. It would only be with the impermissible use of hindsight that it could be alleged that one of ordinary skill in the art would know that locally administering a TRH-containing microstructure to the CNS in a site-specific manner would result in an increase in glutamate and/or aspartate. Accordingly, there is no motivation to combine the cited references in such a way as to arrive at the present invention.

Moreover, Tice et al. discloses microspheres containing a bioactive agent or drug which is implanted in the central nervous system. These microspheres can include microcapsules, nanocapsules, and nanospheres (column 3, lines 7 - 9), which are all characterized as *spherical* particles (column 3, lines 10 - 18).

The invention of Heya et al. is directed to microcapsules containing TRH. The microcapsule of Heya et al. is produced in such a manner that *only* spherical microcapsules are produced. The description of microcapsule manufacture starting on page 3, line 54 through page 5, line 33, includes the use of an oil/water liquid emulsion system. Such a system produces only spherical microparticles (Birnbaum, D.T.; Brannon-Peppas, L. *Microparticle Drug Delivery Systems Drug Delivery Systems in Cancer Therapy*. Brown, D.M., ed.; Totowa: Humana Press Inc., 2003, pg. 118, copy enclosed). Specifically, the oil/water emulsion manufacturing process described in Heya et al. inherently generates spherical microcapsules as a result of the thermodynamically-driven minimization of water caging at the oil-water interface. This process results in a structure that has a minimum three dimensional surface area to volume ratio, commonly known as a sphere. Accordingly, a non-spherical microstructure, as recited in pending claims 1-6, could not be made using the manufacturing processes described in Heya et al.

In contrast, the present invention discloses the use of *non-spherical* microstructures containing TRH. The non-spherical characteristic of the microstructure is essential to the

inventive method of increasing glutamate and/or aspartate release in a CNS locus in a site-specific manner. The present invention is an improvement over the prior art, including Tice et al., because the injectable microspheres:

...are ill-suited to provide sustained drug delivery to central nervous system loci because the microspheres tend to disperse in extracellular cerebrospinal fluid (CSF) and are subject to nonspecific uptake and delivery to more distant sites in the brain by CSF through the circumventricular organs, glia and neurons themselves (page 2, lines 7 – 12 of the present application).

Particularly, microdisks are preferred (page 6, lines 20 - 22). Although the size of the disk can vary, the difference in size of the diameter as compared to the thickness enables the present invention to be made larger than the microspheres of the prior art and avoid the possibility of dispersion in extracellular fluid (e.g., spinal fluid). These are therefore less susceptible to nonspecific uptake and delivery to more distant sites in the brain by CSF, glia and retrogradely by neurons (page 7, lines 9 - 13).

In addition, the shape of the microstructure can affect release of the active therapeutic agent. Non-spherical microstructures of the present invention can be designed so that the rate of change of the surface area change is relatively slow compared to the microspheres of the prior art. This is an improvement over the cited prior art, as spherical particles can have, for example, an increased chance of burst release due to quick surface area erosion (page 9, lines 9 - 14).

Therefore, neither Tice et al. nor Heya et al. teach or suggest use of a *non-spherical* microstructure, as required by pending claims 1-6.

In view of the foregoing, none of the cited references teach or suggest a TRH-containing non-spherical microstructure that effectively increases glutamate and/or aspartate release. As described above, the non-spherical microstructure has advantages over the microspheres disclosed in the prior art, and the combination of the disclosures of Tice et al. and Heya et al. does not yield the benefits of the present invention. Since the cited references, alone or in combination, do not teach or suggest all of the elements of the pending claims, the present invention is not obvious in view of Tice et al. and Heya et al. Consequently, the obviousness rejection is improper and should be withdrawn.

Conclusion

Applicants respectfully submit that the patent application is in condition for allowance. If, in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned agent.

Respectfully submitted,

Kristen J. Harrell

Kristen J. Harrell, Ph.D., Reg. No. 51,783
LEYDIG, VOIT & MAYER, LTD.
Two Prudential Plaza, Suite 4900
180 North Stetson Avenue
Chicago, Illinois 60601-6780
(312) 616-5600 (telephone)
(312) 616-5700 (facsimile)

Date: January 29, 2007

6

Microparticle Drug Delivery Systems

*Duane T. Birnbaum, PhD
and Lisa Brannon-Peppas, PhD*

CONTENTS

- INTRODUCTION
- MICROENCAPSULATION TERMINOLOGY
- PREPARATION OF MICROPARTICLES
- PREPARATION OF BIODEGRADABLE MICROPARTICLES
- PREPARATION OF NONBIODEGRADABLE MICROPARTICLES
- RELEASE BEHAVIOR OF ANTICANCER COMPOUNDS
FROM MICROPARTICLES
- ENCAPSULATION AND IN VITRO RELEASE
- IN VIVO STUDIES
- INDUSTRIAL EFFORTS, PRODUCTS, AND CLINICAL TRIALS
- FUTURE DIRECTIONS
- REFERENCES

1. INTRODUCTION

Tremendous opportunities exist for utilizing advanced drug delivery systems for cancer treatments. One such formulation type that has already begun to fulfill its promise is injectable microencapsulated delivery systems. Biodegradable microspheres containing leutinizing hormone-releasing hormone (LHRH) are already used for treatment of hormone dependent cancers and precocious puberty. This product is the Lupron® Depot and its in vivo results will be discussed later in this chapter. In addition, other in vitro and in vivo results from microparticulate delivery systems for traditional cancer-fighting agents will be described. The more recent developments in gene delivery and utilization of targeted delivery and angiogenic factors will be discussed briefly with an emphasis on the possibilities that have yet to be realized. A more complete analysis of some of these future directions of cancer therapy may be found in Part IV of this volume.

2. MICROENCAPSULATION TERMINOLOGY

First, some of the more basic information on the methods of preparing microparticulate formulations must be discussed, with an emphasis on biodegradable microparticles. The terminology used to describe microparticulate formulations can sometimes be inconsistent and confusing to readers unfamiliar with the field. Essentially, the term "microparticle" refers to a particle with a diameter of 1–1000 µm, irrespective of the precise interior

From: *Drug Delivery Systems in Cancer Therapy*
Edited by: D. M. Brown © Humana Press Inc., Totowa, NJ

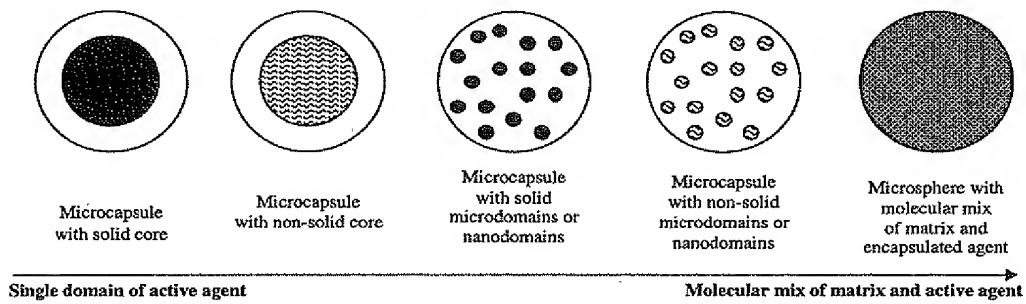


Fig. 1. Variations of microparticle formulations.

or exterior structure. Within the broad category of microparticles, "microspheres" specifically refers to spherical microparticles and the subcategory of "microcapsules" applies to microparticles which have a core surrounded by a material which is distinctly different from that of the core. The core may be solid, liquid, or even gas.

Despite the specific and logical subcategories, many researchers use the terms interchangeably, often to the confusion of the reader. It is usually assumed that a formulation described as a microparticle is comprised of a fairly homogeneous mixture of polymer and active agent, whereas microcapsules have at least one discrete domain of active agent and sometimes more. Some variations on microparticle structures are given in Fig. 1. As the domains and subdomains of active agent within microcapsules become progressively smaller, the microcapsules become microparticles.

3. PREPARATION OF MICROPARTICLES

There are innumerable methods for preparing microparticles for use in applications as diverse as carbonless paper to ion exchange resins to cosmetics to drug delivery. Here, we will concentrate on the materials, biodegradable and nonbiodegradable, which have been studied for drug delivery specifically for cancer treatment.

An overwhelming majority of methods used for encapsulating drugs in a submillimeter spherical polymer matrix involve the use of liquid emulsions. A simple definition of an emulsion as applied to liquids is the dispersion and stabilization of one liquid within another to which it is immiscible. The most common emulsion type is oil-in-water, however, oil-in-oil and multiple emulsions (water-oil-water, oil-oil-water, solid-oil-water, and so on) are used frequently (1-14). There are numerous materials available for creating these emulsions and we discuss a few specific examples here. The main criterion for creating an emulsion is that the dispersed phase (solution containing polymer and drug) must be immiscible (or nearly so) in the continuous phase (external phase containing dissolved surfactant).

4. PREPARATION OF BIODEGRADABLE MICROPARTICLES

4.1. *Poly(lactic-co-glycolic Acid)*

Most systems that use oil-water emulsions to prepare microparticles consist of an organic phase comprised of a volatile solvent with dissolved polymer and the drug to be encapsulated, emulsified in an aqueous phase containing dissolved surfactant (see Fig. 2).

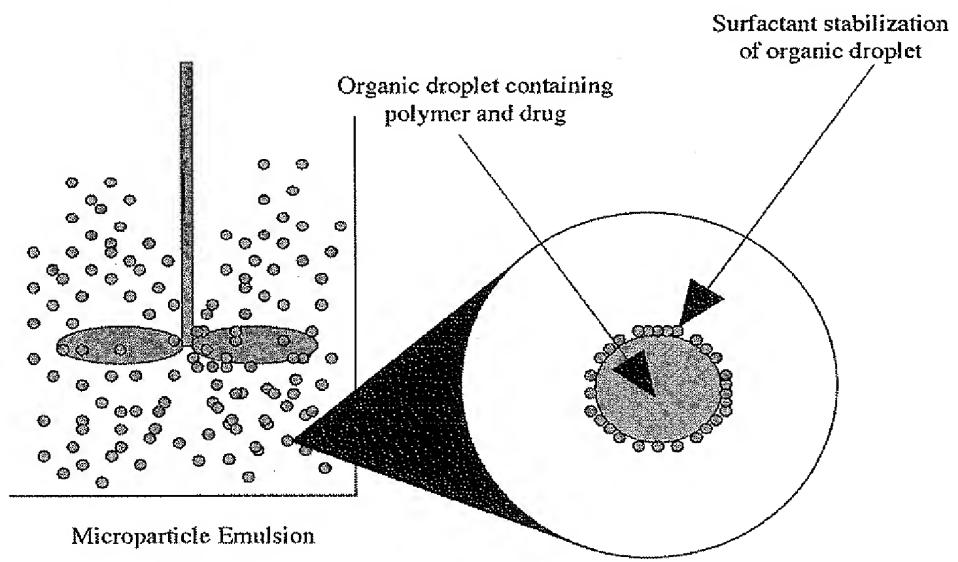
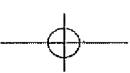


Fig. 2. Encapsulation using oil-in-water emulsion technique.

Two common examples of volatile organic solvents used for the organic-phase solvent are dichloromethane and ethyl acetate. There are numerous examples of biodegradable polymers that can be used for the microparticle matrix, however, polylactic acid (PLA) and the copolymer of lactic and glycolic acid (PLAGA) are the most frequently used due to their high biocompatibility and government approval (15). The PLAGA polymer degrades hydrolytically over time to its monomeric components, which are easily removed from the body through natural life processes. A surfactant is also included in the aqueous phase to prevent the organic droplets from coalescing once they are formed. Once the droplets are formed via physical means, the organic solvent leaches out of the droplet into the external aqueous phase before evaporating at the water-air interface. Emulsions are simply created by using a propeller or magnetic bar for mixing the organic and aqueous phases. The organic-phase solvent should be able to dissolve the polymer up to reasonably high concentrations, preferably in the hundreds of mg/mL, but does not necessarily need to be a good solvent for the drug. The solvent should be completely or almost completely immiscible in water such that a two-phase system can be easily obtained. If the solvent is slightly soluble in water, then steps to control the extraction rate of the solvent into the external aqueous phase need to be considered. A high extraction rate will result in the formation of microparticles with a high porosity that could lead to the untimely and immediate release of drug (16,17). Scanning electron microscopy (SEM) images of microparticles prepared using poly(lactic acid-co-glycolic acid) (PLAGA) and ethyl acetate dispersed in an aqueous PVA solution are shown in Fig. 3. Since ethyl acetate is slightly soluble in water (10% v/v) it partitions upon mixing to the external phase at an increased rate, relative to an immiscible solvent such as dichloromethane, such that the resultant microparticles are highly porous and/or hollow. Saturating the external aqueous phase with ethyl acetate and minimizing its volume will significantly reduce the extraction rate of the ethyl acetate leading to the formation of microparticles with reduced porosity.

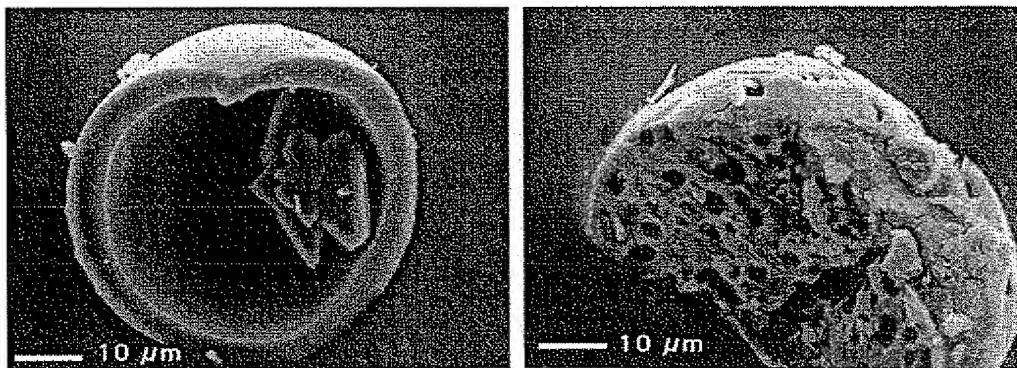


Fig. 3. Hollow and porous microparticles of PLAGA prepared using ethyl acetate and poly(vinyl alcohol) at a magnification of $\times 1250$.

When the drug is not soluble in the organic solvent, it may be encapsulated as a solid provided its form is of small size. Nominally, the size of the drug crystals should be at least an order of magnitude smaller than the desired microparticle diameter in order to avoid large bursts associated with dissolution of larger crystals. Smaller crystals will be more homogeneously distributed throughout the organic droplets created in the emulsion. This results in a solid-in-oil-in-water emulsion (S/O/W) and may be used with any hydrophilic drug (e.g., cisplatin, 5-fluorouracil, and doxorubicin).

The most serious challenge with encapsulating hydrophilic materials is loss of drug to the external aqueous phase during the formation of the microparticles. Along with the loss of drug to the external phase, the remaining material may migrate to the surface of the droplet before hardening. To minimize these problems, the organic droplets should be hardened into microparticles as quickly as possible following their formation. An in-liquid drying process is often used to harden the organic droplets into solid microparticles (see Fig. 4) (18). The method typically involves the use of a viscous organic solution of polymer and drug and a large secondary volume of water that essentially extracts the organic solvent into the external aqueous phase immediately, thus leaving only the microparticle with encapsulated drug. Again, care has to be taken to ensure that high-quality microparticles are produced. Parameters that control the porosity of the resultant microparticles formed from an in-liquid drying/extraction process include the viscosity of the organic solution, volume of organic solvent used, time interval at which the large volume of water is added, and the volume of water used to extract the organic solvent from the particles. The highly viscous dispersed phase serves two purposes. First, the volume of volatile organic solvent is at a minimum, facilitating its quick removal from the droplet. Second, highly viscous material will make the migration of the solid drug particles/crystals to the surface of the droplet more difficult, resulting in a more homogeneous distribution of drug within the microparticle.

As an alternative to S/O/W emulsions, hydrophilic drugs may be encapsulated in a polymer matrix using a multiple water-in-oil-in-water (W/O/W) or oil-in-oil (O/O) emulsions. If a W/O/W emulsion is used, the drug is first dissolved in water and emulsified in an organic phase containing the polymer and a surfactant (see Fig. 5). This emulsion is then dispersed in another aqueous phase containing more surfactant. A complication with this

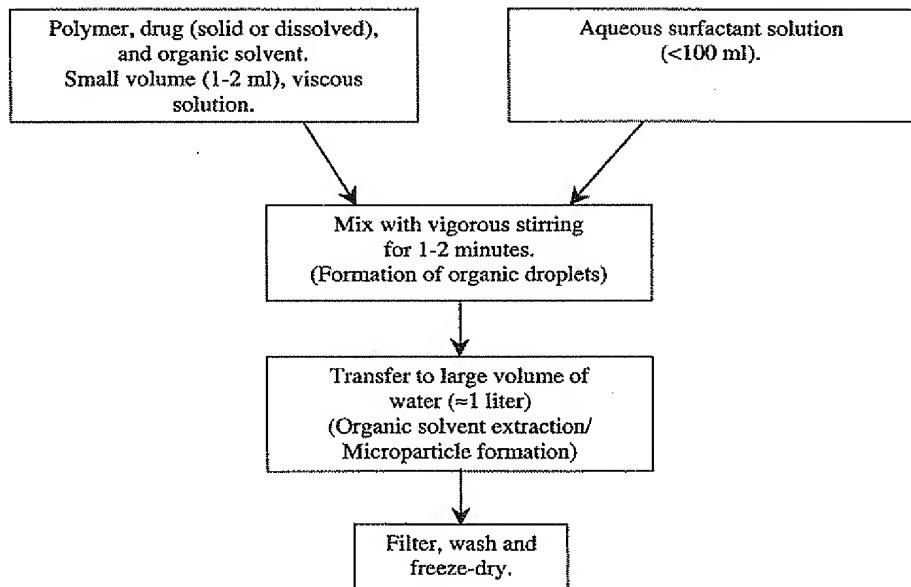
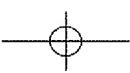


Fig. 4. Schematic diagram of in-liquid drying process for microparticle preparation.

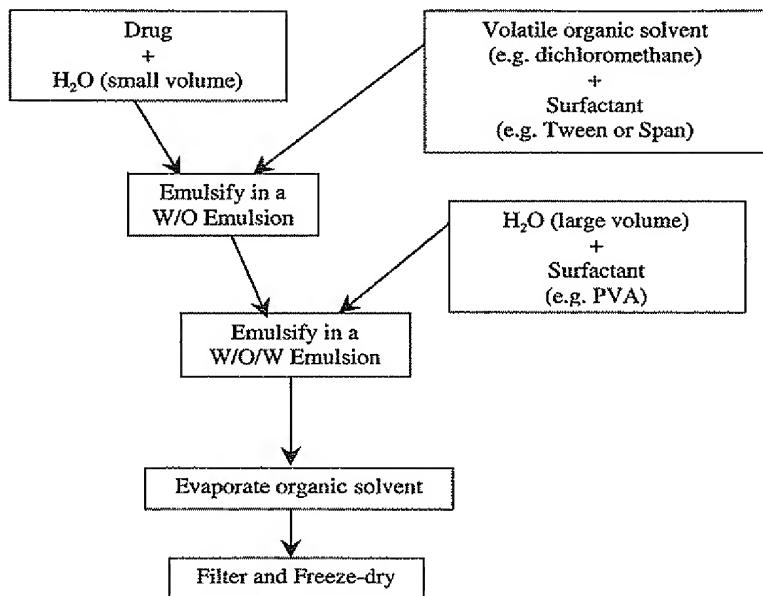


Fig. 5. Schematic diagram of multiple emulsion process for microparticle preparation.

type of emulsion occurs when the inner emulsion is not sufficiently stabilized such that aqueous droplets containing dissolved drug are lost to the external aqueous phase. The choice of surfactants that can be used to stabilize the inner emulsion is limited to materials that will dissolve in the organic solvent. Typically, the fatty acid esters of polyoxyethylene sorbitan or sorbitan are used due to their high solubility in organic solvents and good biocompatibility. With the O/O method, the drug may be suspended or dissolved in the oil phase before being dispersed in another oil phase. One example might use dichloromethane as the solvent for the polymer and dispersant for the drug, and cottonseed oil with the appropriate surfactant added as the external phase in the emulsion. The greatest concern with using highly viscous oil such as cottonseed oil for the external phase is the difficulty in collection and washing of the particles. Filtration of viscous material is significantly more difficult than filtering aqueous suspensions, and washing the microparticles requires the use of yet another organic solvent (typically hexane or heptane).

Encapsulation of hydrophilic drugs may also be achieved by chemically conjugating the drug to the polymer (19–28). The synthesis of such materials will not be discussed here, but typically involves the activation of hydroxyl groups on the polymer, which then react with amino groups located on the drug (24,29). Polyethylene glycol and dextran are often used as the drug carriers; however, it is also possible to conjugate drugs to PLAGA and other biodegradable polymers (30–33). These polymeric “prodrugs” are often administered as intravenous (iv) solutions (if they are soluble in water, dextrans, PEGs, and so on), however, they also may be fashioned into microparticles using the same methods discussed above. The overall drug encapsulation efficiency may not be better than other methods but, assuming all of the drug that ends up attached to the polymer is encapsulated in the microparticles, it allows for easier and more reproducible control over the quantity of drug in the microparticles. Furthermore, because drug will not be released from the microparticles until the hydrolytic degradation of the bond between drug and polymer, there is usually little burst of drug from such particles.

Hydrophobic drugs are typically much easier to encapsulate because they are often highly soluble in the volatile organic solvents used in the formulations and thus lack the thermodynamic drive to partition to the external aqueous phase. Encapsulation efficiencies greater than 90% are typical, with little manipulation of formulation parameters. Challenges arise only when the solubility of the drug is low in the desired dispersed-phase solvent. In these cases, drug loadings may have to be limited to the maximum concentration obtained in the dispersed phase. Alternatively, it may be possible to use a cosolvent system (e.g., dichloromethane and methanol) where the second component is used to increase the concentration of drug in the dispersed phase. If a cosolvent is used for the dispersed phase, it is best if both components are immiscible in water, however, this may not be possible depending on the solubility of the active agent in the various components available. The problems associated with using acetone or methanol as the second component in the dispersed phase is that these are both soluble in water in all proportions. Thus, if acetone or methanol is used to increase the solubility of active agent in the dispersed phase, they may also serve to carry the active agent to the surface of the droplet before partitioning to the aqueous phase. This leaves microparticles with an inordinate amount of active agent at or near the surface, resulting in undesirable release kinetics.

The last method for preparing biodegradable PLAGA microparticles that we will mention here is spray-drying as it is a widely used method in the pharmaceutical industry and has been investigated by several researchers as a method for formulating

biodegradable microparticles (34–40). Although no such studies attempting to encapsulate anticancer agents in biodegradable microparticles using this technique are found in the literature, there is no reason this method could not be used for this purpose. This method for formulating microparticles typically uses drug dissolved or suspended in a polymer solution (either organic or aqueous solvent, depending on the polymer used). This solution/suspension is then fed into the spray-drying apparatus, of which the most important component is the nozzle. Nebulization of the polymer/drug solution may be carried out at the nozzle using different mechanisms (40). Basically, the polymer/drug solution is mixed rapidly with air and forced through a small diameter orifice. Nebulization of the polymer/drug solution occurs at the nozzle and the resultant droplets are very quickly dried by evaporation (under high-pressure air) before collection. Significant advantages of using this technique include high encapsulation efficiencies and no residual surfactant on the surface of the microparticles. There is no external aqueous phase that can act as a sink for the drug, and of course there is no surfactant present anywhere in the formulation. Parameters that can affect microparticle size and morphology are temperature, pressure (air used for drying), nozzle diameter, air/solution volume mixture, and of course, polymer/drug concentrations as is the case for emulsions.

4.2. Albumin-Containing Microparticles

Microparticles of bovine serum albumin (BSA) may be prepared using an emulsion of aqueous BSA in cottonseed oil (41). Sufficient elevation of the temperature of the emulsion will set the BSA microparticles. Any drug may be included, either in solution along with the BSA or in suspension, for water-insoluble drugs. The microparticles are cooled and then washed with ether (or another appropriate solvent) and collected usually by filtration or centrifugation.

To add greater functionality to albumin microspheres, some research groups have included other biodegradable polymers in the microparticles. Specifically, dextran sulfate has been added to these microparticles to add ion-exchange characteristics (42). For this microparticle synthesis, a bovine serum albumin solution was made which also contained sodium dodecyl sulfate and dextran sulfate sodium salt. This phase was emulsified in olive oil, and an aqueous solution of glutaraldehyde was then added to chemically crosslink the albumin. These particular particles were washed with light petroleum, followed by isopropanol followed by distilled water.

4.3. Fibrinogen Microparticles

Microparticles may be prepared from fibrinogen using procedures very similar to those used to make albumin microparticles. One specific method combines fibrinogen and drug in an aqueous solution and then emulsifies this solution in cottonseed oil containing 10% Span 85® (43). This emulsion is added to more cottonseed oil and heated to 100 or 140°C to set the fibrinogen. After 30 min of stirring, the solution is cooled, the particles are washed with ether and dried. Clearly, all of these methods for preparing microparticles of albumin and fibrinogen should be effective for encapsulating water-soluble drugs, which are not proteins, for cancer treatment. Drugs that are more soluble in the oil phase can easily partition out of the microparticles during the stirring step, as the microparticles are in a swollen state during preparation. Also, it is impossible to encapsulate active proteins within these microspheres as the drug will be denatured during the setting or crosslinking step when the microparticles are formed.

5. PREPARATION OF NONBIODEGRADABLE MICROPARTICLES

For nonbiodegradable microparticles to be useful in drug delivery, the active agents must be adsorbed onto the surface of the particles. Such solid particles have been used in specific treatments of embolization to block the blood flow to cancer tissue. Very few permanent microparticle systems have been studied, with the exception of magnetic microparticles that are currently being used by FeRx Incorporated. FeRx uses magnetic targeted carriers as delivery vehicles for the site-specific targeting, retention, and sustained release of cancer-fighting active agents. These carriers approx 0.5 to 5 μm in diameter and are composed of elemental iron and activated carbon. The drugs to be delivered are bound to the surface of the particles, which are then localized using a small externally positioned magnetic field that directs the particles, and the drug adsorbed on them, specifically to the cancerous tissue.

6. RELEASE BEHAVIOR OF ANTICANCER COMPOUNDS FROM MICROPARTICLES

This and following sections present predominately a review of the work published in the last five years, categorized according to the active agent being delivered. This direct comparison should help to point out the diverse approaches using microencapsulation techniques that are being investigated and used to control the delivery of cancer-treating drugs. Of course, with the approval already in place for PLAGA for *in vivo* applications, many different research groups have focused their work on that polymer family. This chapter will not be addressing formulations that are either liposomes, micelles, or polymer conjugates as those topics are covered elsewhere in this volume.

7. ENCAPSULATION AND IN VITRO RELEASE

7.1. *Hydrophilic Drugs*

The encapsulation of hydrophilic compounds such as doxorubicin and 5-fluorouracil within hydrophobic biodegradable polymers presents a serious challenge because of the thermodynamic drive of these drugs to partition to the aqueous external phase in an O/W emulsion. Some methods used to alleviate these challenges include using O/O emulsions to avoid the use of water, in-liquid drying processes to harden the microparticles quickly, and covalently attaching the drug to the polymer used for the biodegradable matrix before preparing the microparticles.

7.1.1. DOXORUBICIN

Doxorubicin is widely used for the treatment of many types of cancer and is moderately hydrophilic, with a solubility of the hydrochloride salt in water of approx 10 mg/mL. Doxorubicin has been encapsulated in a wide range of microparticles prepared from albumin, albumin-dextran sulfate, fibrinogen, and poly(lactic acid) (41–44). Methods involved W/O emulsion for fibrinogen or albumin microparticles, O/W emulsion for PLA, and an ion-exchange W/O method for albumin-dextran sulfate. It should also be possible to encapsulate doxorubicin using W/O/W or S/O/W methods.

Albumin particles with an average diameter of approx 1 μm encapsulating doxorubicin were prepared using a W/O emulsion (41). Encapsulation efficiencies were low (23%) and the resulting drug loading was slightly over 2% by mass. Because cottonseed

oil was used as the external phase, very high encapsulation efficiencies would have been expected. The authors did not discuss the unusually low encapsulation efficiency of the doxorubicin in their formulation. The *in vitro* release of doxorubicin from these particles in tris buffer was biphasic with approx 1/3 of the drug released in the first 6 h, followed by the release of the remaining drug over the next 90 h. The initial burst of drug is typically due to surface-bound drug. The relatively short release time (approx 4 d) is a function of the microparticle size (1 μm) and the nature of the material used for the microparticle matrix, in this case, water-soluble bovine serum albumin (BSA).

Another microparticle formulation containing doxorubicin and prepared from bovine serum albumin using a W/O emulsion resulted in particles with a size range of 20–60 μm (42). Dextran sulfate was also encapsulated into the albumin microparticles. In this case, doxorubicin was loaded after the preparation of the microparticles. The particles were first swollen with ethanol, and water was then added to a doxorubicin solution. After 1 h, essentially all the drug was taken up by the particles via an ion-exchange mechanism with the sulfate groups of the derivatized dextran already encapsulated in the albumin microparticles. Drug loadings were as high as 50% by mass as a result of the very efficient ion exchange process. Release of doxorubicin in phosphate buffered saline (PBS) was nearly constant and lasted for approx 10 h. The release time was doubled by including iron in the formulation, which is known to complex with doxorubicin.

Although these microparticles containing albumin and dextran are considerably larger than those previously mentioned containing only albumin, the total time required to release the doxorubicin is considerably less (90 h vs 10 h). This is owing to the method used to encapsulate the drug. The ion-exchange method encapsulates the doxorubicin after the microparticles are formed. Therefore, the drug diffuses into the microparticles and is held there only by the strength of the ionic interaction with the sulfate groups of the derivatized dextran. Consequently, the release of doxorubicin under sink conditions will be governed primarily by this ionic interaction because diffusion is quite rapid. The release of doxorubicin from the smaller diameter microparticles mentioned earlier will be governed by degradation and diffusion as the drug was encapsulated at the time of microparticle formation.

Fibrinogen particles containing doxorubicin have also been prepared from a W/O emulsion (43). Again, the external oil phase used was cottonseed oil. Drug loadings were approx 10% with encapsulation efficiencies of 40–45%. Particle diameters averaged 2–3 μm with all particles under 10 μm in diameter. Sustained release of the drug had occurred for at least 7 d when the studies were stopped after 10–20% of the drug had been released. There was no initial burst from the fibrinogen microspheres, indicating that the fibrinogen was a very effective encapsulant for the doxorubicin. The fibrinogen microparticles appear to swell much less than albumin microparticles, even when preparation conditions are similar. The degradation, both *in vitro* and *in vivo*, may also be slower for the fibrinogen.

Doxorubicin has also been encapsulated in varying molecular weight oligomers of polylactic acid with particle diameters of approx 100 μm using an O/W emulsion (44). Drug loadings ranged from 0.5 to 2.5% by mass with encapsulation efficiencies of 30–90%. The relatively high encapsulation efficiencies were attributed to the doxorubicin being slightly soluble in the dichloromethane (2–3 $\mu\text{g}/\text{mL}$) that was used as the organic solvent for the dispersed phase. Thus, the drug will partition to the external

aqueous phase at a decreased rate relative to a formulation in which doxorubicin is insoluble in the organic solvent. There was also a strong correlation between doxorubicin encapsulation efficiency and molecular weight of PLA used for the matrix. The lower molecular weight PLA used resulted in higher doxorubicin encapsulation efficiencies. Sustained release of drug was observed in tris buffer for a period of several days with an initial burst that increased with increased drug loadings.

7.1.2. CISPLATIN

Significant efforts have also focused on the encapsulation of cisplatin in biodegradable microspheres. Cisplatin is slightly soluble in water (1 mg/mL), making it a candidate for S/O/W and W/O/W emulsions. Because it is less soluble in water than doxorubicin, encapsulation efficiencies are typically higher when using an aqueous external phase in the emulsion. Cisplatin has been encapsulated in PLAGA/PLA microparticles by a number of research groups (44–51). Particle size ranges have varied between 1 and 300 μm with high encapsulation efficiencies (> 90%). However, depending on the type of emulsion used, a large burst was often observed in the in vitro release profiles. Release times, in vitro, vary between a few days to months depending on the diameter of the microparticles and the molecular weight of the polymer used.

Cisplatin encapsulated in PLA microspheres and prepared using an O/O method produced particles with a diameter of 100 μm (48). Dimethyl formamide and castor oil were used for the dispersed and external-phase solvents, respectively. The microparticles contained 4% drug by mass and released their contents in vitro within 3 d. This is an unexpectedly fast release considering the diameter of the spheres involved. The quick release of cisplatin from these microparticles was attributed to the burst effect from surface-bound drug, suggesting that all encapsulated cisplatin was at or near the surface of the microparticles. Similar results have been obtained by other researchers when encapsulating cisplatin in PLAGA microparticles using an O/O emulsion (44,47–49). High encapsulation efficiencies and drug loadings are achieved, but the in vitro release displays large bursts within the first few hours that increase with the drug loading. These initial bursts of drug may be as high as 80% of the total amount of drug encapsulated in the microparticles. As the amount of the drug in the particles increased so did the burst, and the subsequent duration of the controlled release decreased significantly.

More satisfactory results were obtained by using a more traditional S/O/W method with dichloromethane as the dispersed-phase solvent and a PVA solution for the external phase (45). Microparticles of a diameter between 100–200 μm exhibited a slow sustained release of drug in vitro for a period of 60 d before the remaining drug was released over a period of approx 1 wk. The release of drug in this manner is consistent with a diffusion mechanism that is followed by the degradation of the polymer to a point where the microparticles release the remaining drug. Similar results have been obtained with 5-fluorouracil encapsulated in PLAGA microspheres, as discussed in the following section. Other studies have also used the S/O/W emulsions to encapsulate cisplatin in PLAGA microparticles with excellent results showing high encapsulation efficiencies and little or no burst (46,50,51).

7.1.3. 5-FLUOROURACIL

Another slightly hydrophilic drug widely used in cancer treatments, with a solubility in water of approx 1 mg/mL, is 5-fluorouracil (5-FU). Microparticles (3–6 μm) prepared from PLA using a S/O/W emulsion contained 5–15% 5-FU by mass and released

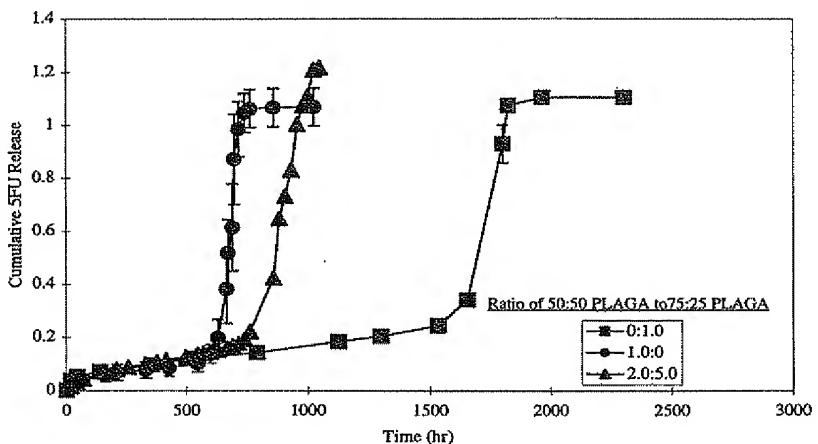
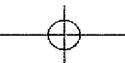


Fig. 6. In vitro drug delivery in buffered saline at 37°C for 5-fluorouracil from microparticles prepared from various ratios of 50:50 PLAGA (mol wt: 13,000) and 75:25 PLAGA (mol wt: 65,000).

drug in vitro over a period of about 5 d (52). There was a substantial burst (20–40% of encapsulated drug) from these particles that increased with the initial drug loading. Microparticles using cross-linked chitosan and containing 5-FU have been prepared using a W/O emulsion (53). Drug loadings were as high as 20% by mass. However, these particles released the drug in saline in just a few hours despite their relatively large diameters (490–760 μm), indicating that any crosslinking was not effective in controlling the release of the 5-FU.

As with cisplatin, it is possible to encapsulate the hydrophilic drug 5-FU with high efficiency and drug loadings and still obtain a desirable release profile with little to no burst and relatively constant release. In our research group, we have prepared 5-FU microparticles from high and low molecular weight PLAGAs as well as a mixture of molecular weights. A S/O/W emulsion was used in conjunction with a highly viscous organic phase and an in-liquid drying process. The resulting microparticles were 50–60 μm in diameter with encapsulation efficiencies as high as 75% and drug loadings as high as 25%. Release profiles in buffered saline from PLAGA microparticles are shown in Fig. 6. The initial release is slow and sustained with no burst and lasts three or more weeks, depending on the molecular weight of the PLAGA samples used, with higher molecular weight polymers yielding formulations with longer controlled-release duration. After the polymer degradation reaches a critical phase, the remaining drug is quickly released over a period of about 1 wk. Thus the release is controlled by both diffusion and polymer hydrolysis rates, resulting in a biphasic release profile. The time lag between the slower release phase and the faster release phase can be controlled by using different molecular weight PLAGA or a blend of PLAGA polymers with differing molecular weights. Because higher molecular weight PLAGA will hydrolyze at a slower rate, the initial slow-release phase will last longer when using higher molecular weight PLAGA, either alone or in a blend. The release profile can be made monophasic by including low molecular weight PLAGA and hydrophilic polyethylene glycol (PEG) in the formulation, as shown in Fig. 7. At

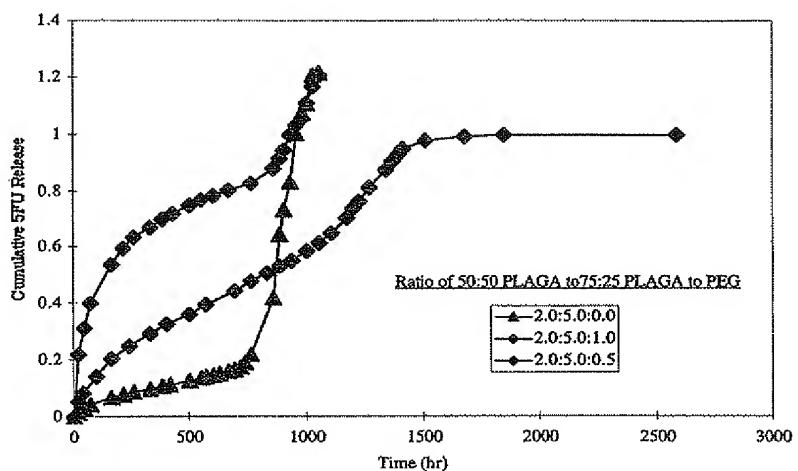


Fig. 7. In vitro drug delivery in buffered saline at 37°C for 5-fluorouracil from microparticles prepared from various ratios of 50:50 PLAGA (mol wt: 13,000), 75:25 PLAGA (mol wt: 65,000), and PEG (mol wt: 6,000).

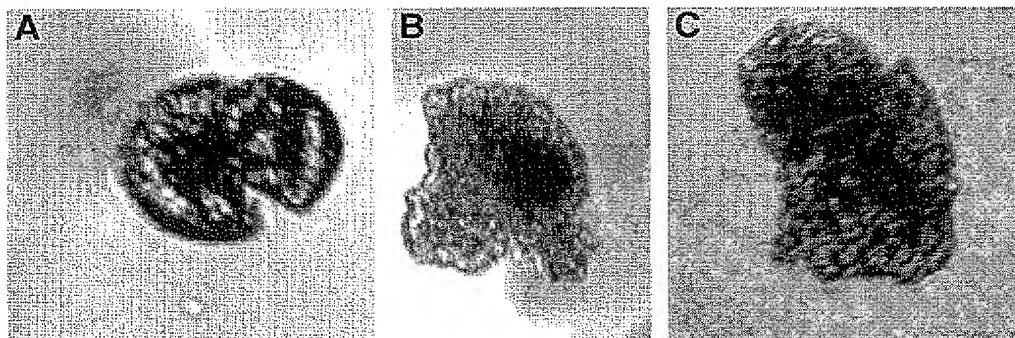


Fig. 8. Microscopic images of PLAGA microparticles containing 5-fluorouracil after 21 days of degradation in phosphate buffered saline. The relative ratios of 50:50 PLAGA (mol wt: 13,000) to 75:25 PLAGA (mol wt: 65,000) to PEG (mol wt: 6000) are (A) 2:5:0; (B) 2:5:0.5; and (C) 2:5:1.

approx 3.5% PEG (mol wt: 6000), the microparticles release 5-FU at a nearly constant rate for the entire profile. The hydrophilic PEG will release quickly (relative to hydrolysis rate of PLAGA) through diffusion, creating a more porous microparticle relative to those without PEG, enabling the encapsulated 5-FU to diffuse out of the microparticle at a higher rate. This combination of release behaviors then gives a monophasic release profile. Some microscopic images of these microparticles during degradation are shown in Fig. 8. These figures show that, while particles composed of PLAGA remain essentially spherical only until after all drug delivery has been completed, those microparticles containing PEG show significant physical changes and degradation during the drug delivery phase.

7.1.4. LEUTINIZING HORMONE RELEASING HORMONE (LHRH)

Proteins present a unique problem when being encapsulated in polymer matrices using the techniques described above. Leutinizing hormone releasing hormone (LHRH) is not only one of the best known peptides studied for controlled-release formulations from biodegradable microparticles but it also is one of the very few commercially available products using this technology (Lupron® Depot) (54,55). LHRH in cancer treatment is used to suppress the production of sex hormones that lead to hormone-dependent cancers. Proteins in general are notorious for chemical instabilities, especially when exposed to organic solvents. Thus, proteins and peptides are almost always formulated into microparticles using a W/O/W emulsion. The use of an inner aqueous phase serves to protect the dissolved protein from the harsh environment of the organic solvent.

LHRH has been encapsulated in PLAGA microparticles using a W/O/W emulsion (56) and by cryogenic grinding of extruded PLG containing homogeneously distributed peptide (57). Microparticles prepared using a W/O/W emulsion containing 75:25 PLAGA (mol wt: 14,000) and 5% LHRH released in vitro for several weeks (>4) with no initial burst of hormone. That a water-soluble drug could be efficiently entrapped in a PLAGA microparticle and display no initial burst using the W/O/W method is somewhat unusual and very encouraging for researchers in the field. For example, numerous research groups have used the W/O/W method to encapsulate various proteins (e.g., BSA) and the in vitro release typically displays a moderate to large burst. That LHRH has both high encapsulation efficiency and no initial burst has been attributed to the formation of a micelle-like structure between the PLAGA chains and the drug (57). The release of the hormone is then strictly regulated by polymer degradation rather than diffusion.

7.2. *Hydrophobic Drugs*

Hydrophobic drugs often present less of a challenge to formulate in slowly degradable microparticle systems, relative to hydrophilic drugs, as they are often soluble in the organic solvents used, but are insoluble in water. Some hydrophobic anticancer agents that have been encapsulated in biodegradable microparticles include taxol, aclaracinomycin, and camptothecin (water-insoluble forms) (58–65).

Anticancer agents such as taxol, aclaracinomycin, camptothecin, and related analogs have been successively encapsulated in PLAGA microparticles with high efficiency (> 90%) (58,61,63). The maximum amount of drug encapsulated in the microparticles typically depends on its solubility in the organic solvent used in the formulation. Taxol is now a common anticancer agent used against a wide variety of solid tumors including breast and ovarian cancers. It is insoluble in water and has a limited solubility in ethanol. Therefore, commercial formulations of taxol use a 50:50 mixture of ethanol and Cremophor® EL (polyethoxylated castor oil) which are then designed to be diluted in saline or other intravenous infusion solutions. Alternative dosage forms are desired not only for the controlled release, but also to reduce adverse reactions to the relatively large amount of Cremophor EL used in the commercial formulation (63).

Taxol has been encapsulated in PLAGA microparticles of varying LA/GA ratios using very simple O/W emulsions (63). The authors used dichloromethane as the solvent for both PLAGA (mol wt: 10,000) and taxol. A 4% gelatin solution was used as the continuous phase with simple mechanical mixing. An encapsulation efficiency of

98% from microparticles with an average diameter of 30 μm was achieved using 75:25 PLAGA with no additional additives. The *in vitro* release displayed a slow-sustained release of taxol with no initial burst. In fact, the release was so slow that isopropyl myristate was added to change the microparticle matrix to allow the formation of channels that would allow for faster diffusion of taxol from the microparticle. These results are not unexpected considering the hydrophobic nature of the drug and the immiscible nature of the solvents used for both phases of the emulsion. Thermodynamically, taxol must remain solvated in the dichloromethane until which time the solvent is completely removed and, thus, the drug is homogeneously encapsulated in the newly formed microparticle. Because the release of taxol from PLGA microparticles is typically quite slow, the most significant obstacle in formulating these microparticles is obtaining a sustained release of therapeutic levels of drug. Thus, additives such as isopropyl myristate, sucrose, and the use of PLAGAs of varying molecular weight and hydrolytic degradation rates have been investigated as a means of accelerating the release of taxol (63,64).

Camptothecin (CPT) is a promising anticancer agent being investigated in the treatment of a wide variety of tumors (59). Because of the low solubility of camptothecin in water (3.8 $\mu\text{g/mL}$) and dichloromethane (10 $\mu\text{g/mL}$) the drug poses a somewhat different challenge with regard to formulating microparticles for controlled release. There are basically two options when formulating such compounds through the use of emulsions: encapsulate the drug in a solid form using a S/O/W emulsion or use a cosolvent system that will dissolve both polymer and drug to desired levels. Dimethylformamide (DMF) is an excellent solvent for CPT and is also highly miscible with dichloromethane, therefore, it serves well as the second solvent component for the dispersed phase in a microparticle emulsion. However, DMF is also miscible with water, an undesirable property for any component of the dispersed-phase solvent. The authors' results are as expected: higher encapsulation efficiencies with decreased DMF volume and higher concentration of PLAGA in the dispersed-phase solvent. Drug release profiles are also consistent with expectations; the burst was minimized by decreasing the volume of DMF and increasing the concentration of PLAGA.

8. IN VIVO STUDIES

Although limited in terms of the number of studies reported in the public literature, data for the *in vivo* release of anticancer agents encapsulated in biodegradable microparticles demonstrates advantages over administration of the free drug. Doxorubicin, 5-FU, cisplatin, irinotecan (synthetic derivative of camptothecin), and LHRH have been administered into tumor-bearing rats and/or mice using biodegradable microparticles (43,48,49,52,66-68).

Fibrinogen microparticles (average diameter, 2-3 μm) containing varying amounts of doxorubicin were injected into mice inoculated with Ehrlich ascites carcinoma cells, and survival times recorded versus those of mice treated with a doxorubicin solution (43). Relative to the control animals, where half of the mice had died at 20 d (with no survivors at 29 d), the injection of doxorubicin solution decreased the lifespan of the mice, with half dead at 10 d (with no survivors at 28 d), whereas injection of fibrinogen microspheres containing doxorubicin significantly increased the lifespan of the mice, with more than half surviving longer than 60 d. These results were obtained for doxorubicin administered at a level of 13.7 mg/kg. The authors interpreted these results as

an indication that biodegradable fibrinogen microspheres containing doxorubicin could be used to administer increased amounts of doxorubicin with decreased toxicity, thus reducing systemic side effects caused by the drug.

PLAGA microparticles containing 5-FU have been stereotactically implanted in the brains of rats with malignant gliomas to test their toxicity and efficacy (66). Similar microparticles (diameter, 3–6 μm) containing 5-FU were also injected into the tail veins of mice in order to measure the *in vivo* distribution of the drug particles (52). Stereotactically implanted microparticles effectively decreased the mortality of malignant tumor-bearing rats. However, the decreased mortality was found to be statistically significant only for the slow-releasing microspheres where the 5-FU was released over 18 d, as opposed to fast-releasing microspheres where the 5-FU was released over 3 d. Microparticles containing 5-FU, which were injected into the tail veins of mice, were found primarily in the lungs and liver within 24 h postinjection.

Microparticles containing cisplatin have been studied *in vivo* as a general aid to understanding the effect on surrounding muscle tissue in rabbits and mice as well as their ability to increase the survival time of tumor-bearing mice (48,49). For mice inoculated intraperitoneally with P815 mastocytoma cells, the mean survival times were compared to those mice injected with (1) cisplatin containing microspheres, (2) cisplatin solutions (free cisplatin), (3) blank microspheres, and (4) phosphate-buffered saline (PBS). Several different doses of cisplatin were also studied (49). All tumor-bearing mice injected with blank microspheres or PBS died within 20–30 d. At lower levels of cisplatin (<200 μg), the mice died from the tumors within 40–60 d and at higher levels of cisplatin (>350 μg), the mice died of drug toxicity in less than 7 d. For cisplatin-containing microspheres, the mice survived for an average as long as 140 d. From this study it seems clear that using a microparticle controlled-release formulation is advantageous in terms of relative toxicity and efficacy of the drug formulation.

The *in vivo* effectiveness of the release of irinotecan hydrochloride, a semisynthetic derivative of camptothecin, was also studied for microparticle formulations of PLA as well as 75:25 and 50:50 PLAGA (67). These studies, where mice were transplanted intraperitoneally with sarcoma 180 cells, showed a mean survival time for the control groups of approx 11 d. Treatment was begun 3 d after inoculation; irinotecan hydrochloride solutions at 50 mg eq/kg, 100 mg eq/kg, and 250 mg eq/kg showed mean survival times of 22, 16, and 16 d, respectively. Microparticle formulations prepared with PLA and at the same equivalent drug dosages showed mean survival times of 20, 16, and 32 d, respectively. Formulations at the 100 mg eq/kg level for 75:25 PLAGA and 50:50 PLAGA showed 20 and 24 d mean survival time. These studies demonstrated that, for the microparticle formulations to be significantly more effective in terms of survival time than the drug solution, the drug loading must be at least 250 mg eq/kg.

9. INDUSTRIAL EFFORTS, PRODUCTS, AND CLINICAL TRIALS

The one product currently on the market which uses microparticle formulations to treat cancer is the Lupron[®] Depot from TAP Pharmaceuticals (69). The Lupron[®] Depot is available in 4-, 3-, and 1-mo formulations which are approved in the United States for palliative treatment of advanced prostate cancer. These formulations contain 30, 22.5, and 7.5 mg of leuprolide acetate, respectively. In an open-label, noncomparative, multicenter clinical study of the 4-mo formulation, 49 patients with stage D2 prostatic adenocarcinoma (with no prior treatment) were enrolled. The objectives were to deter-

mine whether this 30 mg depot formulation, injected once every 16 wk, would reduce and maintain serum testosterone levels at castrate levels (less than or equal to 50 ng/dL). In the majority of patients, testosterone levels increased 50% or more above the baseline during the first week of treatment, however, these levels were subsequently suppressed to castrate levels within 30 d of the first injection in 94% of patients and within 43 d in all 49 patients during the initial 32-wk treatment period.

Lupron® Depot, available in formulations of 11.25 mg (3-mo dose) and 3.75 mg (1 mo dose), is also used with iron before surgery in the management of endometriosis and to treat anemia caused by fibroid tumors in the uterus when iron alone is not effective. For precocious puberty, the dosage is 7.5, 11.25, or 15 mg once per month, depending upon the weight of the child. Lupron® Depot has also been reported to have been used, quite effectively in some cases and questionably in others, in "off-label" situations such as infertility treatments for humans and adrenal tumor treatment in ferrets.

Although there are a vast number of clinical trials addressing cancer treatment at any time, very few of these are testing microencapsulated formulations. There are at least 1200 open clinical trials in the United States at any time and a recent review of those listed with the National Center Institute revealed only one to be testing a microparticulate delivery system for controlled drug delivery. Far more address the use of liposomal delivery systems, which are more widely used for cancer treatment than microparticulate systems. One clinical trial mentioned a study with PEGylated interferon, with a few underway with other pro-drug types of targeted delivery systems.

Although there are assuredly a number of clinical trials under way and not overly publicized, we will mention just a few here. The work that FeRx has done with magnetic targeted carriers has already been mentioned earlier in this chapter. The company also conducted a Phase I/II clinical trial in 1999 for delivery of doxorubicin for treatment of patients with primary liver cancer (70). Paragon Medical introduced SIR-Spheres® in 1997, which are radioactive particles that are placed in liver cancers. The formulation is marketed in Australia, New Zealand, and Asia, with additional human studies being conducted in Hong Kong, Australia, and New Zealand (71). Another set of clinical trials scheduled recently were to examine the treatment of human papillomavirus-associated cervical dysplasia, which can progress to cervical cancer. Zycos, Inc., and Chesapeake Biological Laboratories are collaborating on Biotope CD™, a PLAGA-based delivery system to deliver DNA-based drugs with increased potency (72,73). Specifically for the cervical application, their plasmid DNA is encoded with disease-specific epitopes, which are designed to program cytotoxic Tcells to recognize and destroy the targeted disease.

10. FUTURE DIRECTIONS

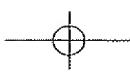
Because it is clear that there is a great deal of potential for the use of microparticulate drug delivery formulations to treat cancer, only a few of these formulations have progressed enough in human studies to have proven their worth both in enhancing the efficacy of the drugs being delivered and in minimizing the undesirable side effects of traditional chemotherapy. Within the next 5–10 yr, we should certainly see some of the formulations currently in laboratories progress to the clinical setting and perhaps to a large number of cancer patients whose lives will be improved by using these advanced formulations. It should be emphasized that microparticulate formulations that provide controlled delivery can provide more than just a better-regulated chemotherapy regimen. They may also deliver cell-specific drugs, based on biotechnology and DNA,

directly to the site of interest. This element of intelligent engineering is present to a smaller degree in liposomal formulations but, especially in biodegradable particles, its promise has yet to be realized or even understood.

REFERENCES

1. Schugens C, Laruelle N, Nihant N, Grandfils C, Jerome R, Teyssie P. Effect of the emulsion stability on the morphology and porosity of semicrystalline poly l-lactide microparticles prepared by W/O/W double emulsion-evaporation. *J Controlled Release* 1994; 32:161-176.
2. Tyle P. Sustained release growth hormone compositions for parenteral administration and their use, Europe Patent 0 278 103, assigned to American Cyanamid Co.
3. Tyle P. Sustained release growth hormone compositions for parenteral administration and their use, US Patent 4857506, assigned to American Cyanamid Co.
4. Viswanathan NB, Thomas PA, Pandit JK, Kulkarni MG, Mashelkar RA. Preparation of non-porous microspheres with high entrapment efficiency of proteins by a (water-in-oil)-in oil emulsion technique. *J Controlled Release* 1999; 58:9-20.
5. Yan C, Resau JH, Heweston J, West M, Rill WL, Kende M. Characterization and morphological analysis of protein-loaded poly(lactide-co-glycolide) microparticles prepared by water-in-oil-in-water emulsion technique. *J Controlled Release* 1994; 32:231-241.
6. Prankerd RJ, Stella VJ. The use of oil-in-water emulsions as a vehicle for parenteral administration. *J Parent Sci Technol* 1990; 44:139-149.
7. Malamataris S, Avgerinos A. Controlled release indomethacin microspheres prepared by using an emulsion solvent-diffusion technique. *Int J Pharmaceut* 1990; 62:105-111.
8. Kawashima Y, Niwa T, Handa T, Takeuchi H, Iwamoto T, Itoh K. Preparation of controlled-release microspheres of ibuprofen with acrylic polymers by a novel quasi-emulsion solvent diffusion method. *J Pharmaceut Sci* 1989; 78:68-72.
9. Grandfils C, Flandroy P, Nihant N, Barbette S, Jerome R, Teyssie P, Thibaut A. Preparation of Poly(D,L) lactide microspheres by emulsion-solvent evaporation, and their clinical applications as a convenient embolic material. *J Biomed Mater Res* 1992; 26:467-479.
10. Deng XM, Li XH, Yuan ML, Xiong CD, Huang ZT, Jia WX, Zhang YH. Optimization of preparative conditions for poly-DL-lactide-polyethylene glycol microspheres with entrapped vibrio cholera antigens. *J Controlled Release* 1999; 58:123-131.
11. Couvreur P, Blanco-Prieto MJ, Puisieux F, Roques B, Fattal E. Multiple emulsion technology for the design of microspheres containing peptides and oligopeptides. *Adv Drug Deliv Rev* 1997; 28:85-96.
12. Conway BR, Alpar HO. Double emulsion microencapsulation of proteins as model antigens using polymers: effect of emulsifier on the microsphere characteristics and release kinetics. *Europ J Pharmaceut Biopharmaceut* 1996; 42:42-48.
13. Candau F, Zekhmini Z, Heatley F, Franta E. Characterization of poly(acrylamide-co-acrylates) obtained by inverse microemulsion polymerization. *Colloid Polymer Sci* 1986; 264:676-682.
14. Ekman B, Sjoholm I. Improved stability of proteins immobilized in microparticles prepared by a modified emulsion polymerization technique. *J Pharmaceut Sci* 1978; 67:693-696.
15. Vert M, Schwach G, Engel R, Coudane J. Something new in the field of PLA/GA bioresorbable polymers? *J Controlled Release* 1998; 53:85-92.
16. Jeyanthi R, Thanoo BC, Metha RC, DeLuca PP. Effect of solvent removal technique on the matrix characteristics of polylactide/glycolide microspheres for peptide delivery. *J Controlled Release* 1996; 38:235-244.
17. Li W-I, Anderson KW, Mehta RC, DeLuca PP. Prediction of solvent removal profile and effect on properties for peptide-loaded PLGA microspheres prepared by solvent extraction/evaporation Method. *J Controlled Release* 1995; 37:199-214.
18. Thies C. Formulation of degradable drug-loaded microparticles by in-liquid drying processes, in *Microcapsules and Nanoparticles in Medicine and Pharmacy* (Dunbrow M, ed). CRC, Boca Raton, FL, 1992, pp 47-71.
19. Takakura Y, Hashida M. Macromolecular carrier systems for targeted drug delivery: pharmacokinetic considerations on biodistribution. *Pharmaceut Res* 1996; 13(6):820-831.
20. Chari RVJ. Targeted delivery of therapeutics: tumor-activated prodrug therapy. *Adv Drug Deliv Rev* 1998; 31:89-104.
21. Kopecek J. Development of tailor-made polymeric prodrugs for systemic and oral delivery. *J Bioactive and Compatible Polymers* 1988; 3:16-26.

22. Mori A, Kennel SJ, Huang L. Immunotargeting of liposomes containing lipophilic antitumor prodrugs. *Pharmaceut Res* 1993; 10:507-514.
23. Nichifor M, Schacht EH. Cytotoxicity and anticancer activity of macromolecular prodrugs of 5-fluorouracil. *J Bioactive and Compatible Polymers* 1997; 12:265-281.
24. Nichifor M, Schacht EH, Seymour LW. Polymeric prodrugs of 5-fluorouracil. *J Controlled Release* 1997; 48:165-178.
25. Ranucci E, Spagnoli G, Latini R, Bernasconi R, Ferruti P. On the suitability of urethane bonds between the carrier and the drug moiety in the poly(ethyleneglycol)-based oligomeric prodrugs. *J Biomater Sci Polymer Edn* 1994; 6:133-139.
26. Ranucci E, Satore L, Peroni I, Latini R, Bernasconi R, Ferruti P. Pharmacokinetic results on naproxen prodrugs based on poly(ethyleneglycol)s. *J Biomater Sci Polymer Edn* 1994; 6:141-147.
27. Steyger PS, Baban DF, Brereton M, Ulbrich K, Seymour LW. Intratumoural distribution as a determinant of tumour responsiveness to therapy using polymer-based macromolecular prodrugs. *J Controlled Release* 1996; 39:35-46.
28. Subr V, Kopecek J, Pohl J, Baudys M, Kostka V. Cleavage of oligopeptide side-chains in n-(2-hydroxypropyl)methacrylamide copolymers by mixtures of lysosomal enzymes. *J Controlled Release* 1988; 8:133-140.
29. Dosio F, Brusa P, Crosasso P, Arpicco S, Cattel L. Preparation, characterization and properties in vitro and in vivo of a paclitaxel-albumin conjugate. *J Controlled Release* 1997; 47:293-304.
30. Yoo HS, Oh JE, Lee KH, Park TG. Biodegradable nanoparticles containing doxorubicin-PLGA conjugate for sustained release. *Pharmaceut Res* 1999; 16(7):1114-1118.
31. Yokoyama M, Fukushima S, Uehara R, Okamoto K, Kataoka K, Sakurai Y, Okano T. Characterization of physical entrapment and chemical conjugation of adriamycin in polymeric micelles and their design for in vivo delivery to a solid tumor. *J Controlled Release* 1998; 50:79-92.
32. Minko T, Kopeckova P, Kopecek J. Comparison of the anticancer effect of free and HPMA copolymer-bound adriamycin in human ovarian carcinoma cells. *Pharmaceut Res* 1999; 16(7):986-996.
33. Omelyanenko V, Kopeckova P, Prakash RK, Ebert CD, Kopecek J. Biorecognition of HPMA copolymer adriamycin conjugates by lymphocytes mediated by synthetic receptor binding epitopes. *Pharmaceut Res* 1999; 16(7):1010-1019.
34. Takeuchi H, Handa T, Kawashima Y. Controlled release theophylline tablet with acrylic polymers prepared by spray-drying technique in aqueous system. *Drug Develop Indust Pharm* 1989; 15:1999-2016.
35. Baras B, Benoit M-A, Youan C, Botti B, Gillard J. Spray-dried polylactide and poly(lactide-co-glycolide) microparticles in controlled oral vaccine delivery. *J Controlled Release* 1997; 48:289-363.
36. Bittner B, Mader K, Li YX, Kissel T. Evaluation of the microenvironment inside biodegradable microspheres from ABA triblock copolymers of poly(l-lactide-co-glycolide) and poly(ethylene oxide) prepared by spray drying. *Int Symp Control Rel Bioact Mater* 1997.
37. Bodmeier R, Chen H. Preparation of biodegradable poly(lactide) microparticles using a spray-drying technique. *J Pharm Pharmacol* 1988; 40:754-757.
38. Freitas C, Muller R. Spray-drying of solid lipid nanoparticles. *Europ J Pharmaceut Biopharmaceut* 1998; 46:145-151.
39. Gander B, Wehrli E, Alder R, Merkle HP. Quality improvement of spray-dried, protein-loaded D,L-PLA microspheres by appropriate polymer solvent selection. *J Microencapsul* 1995; 12:83-97.
40. Giunchedi P, Conte U. Spray-drying as a preparation method of microparticulate drug delivery systems: an overview. *STP Pharma Sci* 1995; 5:276-290.
41. Gupta PK, Hung CT, Perrier DG. Albumin microspheres. I. release characteristics of adriamycin. *Int J Pharmaceut* 1986; 33:137-146.
42. Chen Y, McCulloch RK, Gray BN. Synthesis of albumin-dextran sulfate microspheres possessing favourable loading and release characteristics for the anticancer drug doxorubicin. *J Controlled Release* 1994; 31:49-54.
43. Miyazaki S, Hasiguchi N, Hou WM, Yokouchi C, Takada M. Preparation and evaluation in vitro and in vivo fibrinogen microspheres containing adriamycin. *Chem Pharm Bull* 1986; 34:3384-3393.
44. Wada R, Tabata Y, Ikada Y. Preparation of poly(lactic acid) microspheres containing anti-cancer agents. *Bull Inst Chem Res* 1988; 66:241-250.
45. Spenlehauer G, Vert M, Benoit JP, Boddaert A. In vitro and in vivo degradation of poly(dl-lactide/glycolide) type microspheres made by solvent evaporation method. *Biomaterials* 1989; 10:557-563.
46. Matsumoto A, Matsukawa Y, Suzuki T, Yoshino H, Kobayashi M. The polymer-alloys method as a new preparation method of biodegradable microspheres: principle and application to cisplatin-loaded microspheres. *J Controlled Release* 1997; 48:19-27.



47. Kyo M, Hyon S-H, Ikada Y. Effects of preparation conditions of cisplatin-loaded microspheres on the in vitro release. *J Controlled Release* 1995; 35:73-82.
48. Ike O, Shimizu Y, Wada R, Hyon S-H, Ikada Y. Controlled cisplatin delivery system using poly(d,l-lactic acid). *Biomaterials* 1992; 13:230-234.
49. Itoi K, et al. In vivo suppressive effects of copoly(glycolic/l-lactic acid) microspheres containing cddp on murine tumor cells. *J Controlled Release* 1996; 42:175-184.
50. Spenlehauer G, Vert M, Benoit J-P, Chabot F, Veillard M. Biodegradable cisplatin microspheres prepared by the solvent evaporation method:morphology and release characteristics. *J Controlled Release* 1988; 7:217-229.
51. Spenlehauer G, Veillard M, Benoit J-P. Formation and characterization of cisplatin loaded poly(d,d-lactide) microspheres for chemoembolization. *J Pharmaceut Sci* 1986; 75:750-755.
52. Ciftci K, Hincal AA, Kas HS, Ercan TM, Sungur A, Guven O, Ruacan S. Solid tumor chemotherapy and in vivo distribution of fluorouracil following administration in poly(l-lactic acid) microspheres. *Pharmaceut Develop Technol* 1997; 2:151-160.
53. Akbuga J, Bergisadi N. 5-fluorouracil-loaded chitosan microspheres:preparation and release characteristics. *J Microencapsulat* 1996; 13:161-168.
54. Sharifi R, Ratanawong C, Jung A, Wu Z, Browneller R, Lee M. Therapeutic effects of leuprorelin microspheres in prostate cancer. *Adv Drug Deliv Rev* 1997; 28:121-138.
55. Schally AV, Comaru-Schally AM. Rational use of agonists and antagonists of luteinizing hormone-releasing hormone (lh-rh) in the treatment of hormone sensitive neoplasms and gynaecologic conditions. *Adv Drug Deliv Rev* 1997; 28:157-169.
56. Okada H, Yamamoto M, Heya T, Inoue Y, Kamei S, Ogawa Y, Toguchi H. Drug delivery using biodegradable microspheres. *J Controlled Release* 1994; 28:121-129.
57. Csernus VJ, Szende B, Schally AV. Release of peptides from sustained delivery systems (microcapsules and microparticles) in vivo. *Int J Peptide Protein Res* 1990; 35:557-565.
58. Wada R, Hyon S-H, Ikada Y. Lactic acid oligomer microspheres containing an anticancer agent for selective lymphatic delivery: I. In vitro studies. *J Bioactive and Compatible Polymers* 1988; 3:126-136.
59. Shenderova A, Burke TG, Schwendeman SP. The acidic microclimate in poly(lactide-co-glycolide) microspheres stabilizes camptothecins. *Pharmaceut Res* 1999; 16:241-248.
60. Yokoyama M, Satoh A, Sakurai Y, et al. Incorporation of water-insoluble anticancer drug into polymeric micelles and control of their particle size. *J Controlled Release* 1998; 55:219-229.
61. Shenderova A, Burke TG, Schwendeman SP. Stabilization of 10- hydroxycamptothecin in poly(lactide-co-glycolide) microsphere delivery vehicles. *Pharmaceut Res* 1997; 14:1406-1414.
62. Ertl B, Platzer P, Wirth M, Gabor F. Poly(d,l-lactic-co-glycolic acid) microspheres for sustained delivery and stabilization of camptothecin. *J Controlled Release* 1999; 61:305-317.
63. Wang YM, Sato H, Horkoshi I. In Vitro in vivo evaluation of taxol release from poly(lactic-co-glycolic acid) microspheres containing isopropyl myristate and degradation of the microspheres. *J Controlled Release* 1997; 49:157-166.
64. Vook N, et al. Taxol microencapsulation into biodegradable uncapped poly(lactide-co-glycolide) copolymers. *Int Symp Control Rel Bioact Mater* 1997; 24:881-882.
65. Ruiz JM, Benoit JP. In vivo peptide release from poly(dl-lactic-acid-co-glycolic acid) copolymer 50/50 microspheres. *J Controlled Release* 1991; 16:177-186.
66. Menei P, Boisdror-Celle M, Guy G. Effect of stereotactic implantation of biodegradable 5-fluorouracil-loaded microspheres in healthy and C6 glioma-bearing rats. *Neurosurgery* 1996; 39:117-124.
67. Machida Y, Onishi H, Morikawa A, Machida Y. Antitumour characteristics of irinotecan-containing microspheres of poly-d,l-lactic acid or poly(d,l-lactic acid-co-glycolic acid) copolymers. *STP Pharma Sciences* 1998; 8:175-181.
68. Hutchinson FG, Furt BJA. Biodegradable polymer systems for the sustained release of polypeptides. *J Controlled Release* 1990; 13:279-294.
69. Tap Pharmevauticals Urology, Lupron® Depot, 1999, <http://www.tapurology.com/Html/lupron.htm>.
70. FeRx Incorporated, FeRx Incorporated, 1999, <http://www.ferx.com/home.htm>.
71. Paragon Medical Limited, Publications on SIR-Spheres and Selective Internal Radiation Therapy, 1999; <http://www.pml.com.au/corporate.html>.
72. Zycos, Breakthrough Cancer Drug Manufacturing Alliance, 1999; <http://www.zycos.com/press/release15.html>.
73. Hedley ML, Curley JM, Langer RS. Microparticles for delivery of nucleic acid, USA Patent 5 783 567, July 21, 1998; assigned to Pangaea Pharmaceuticals.